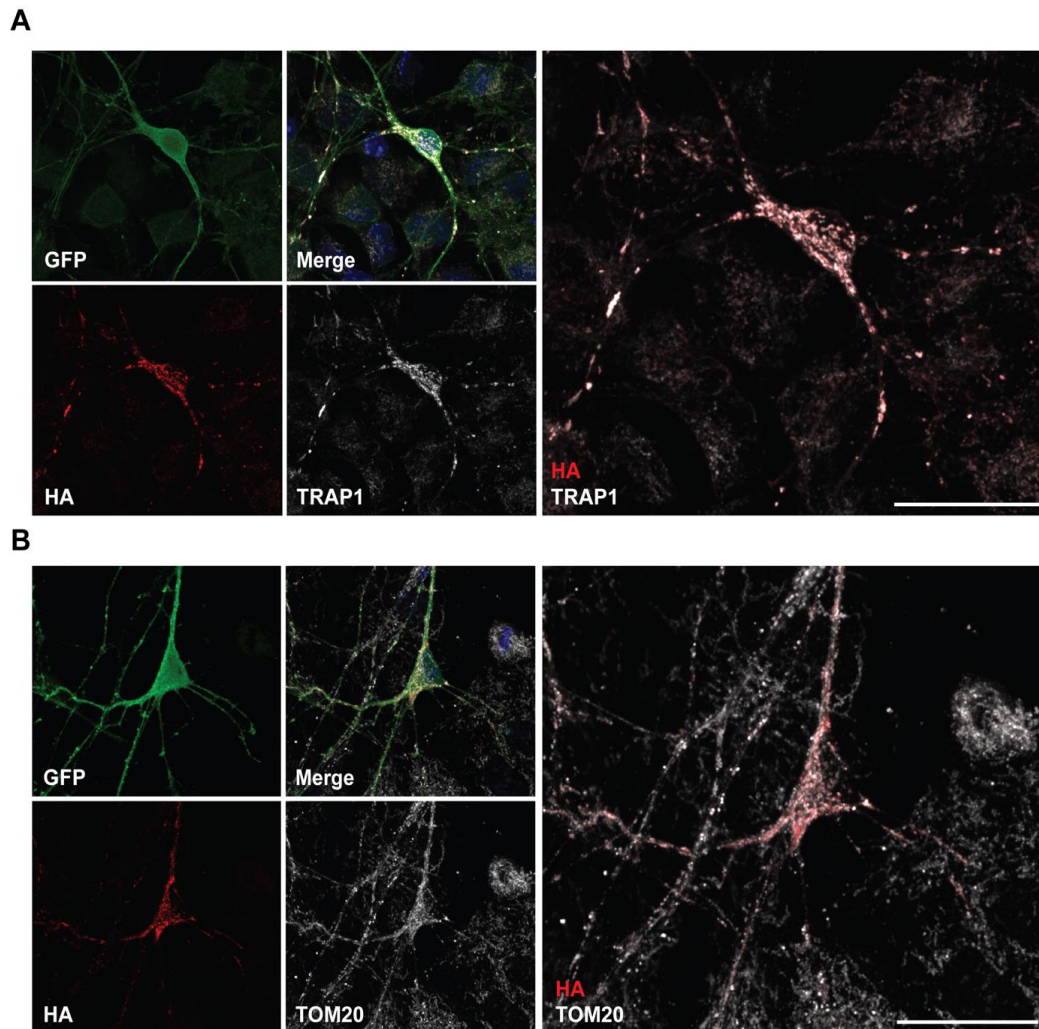
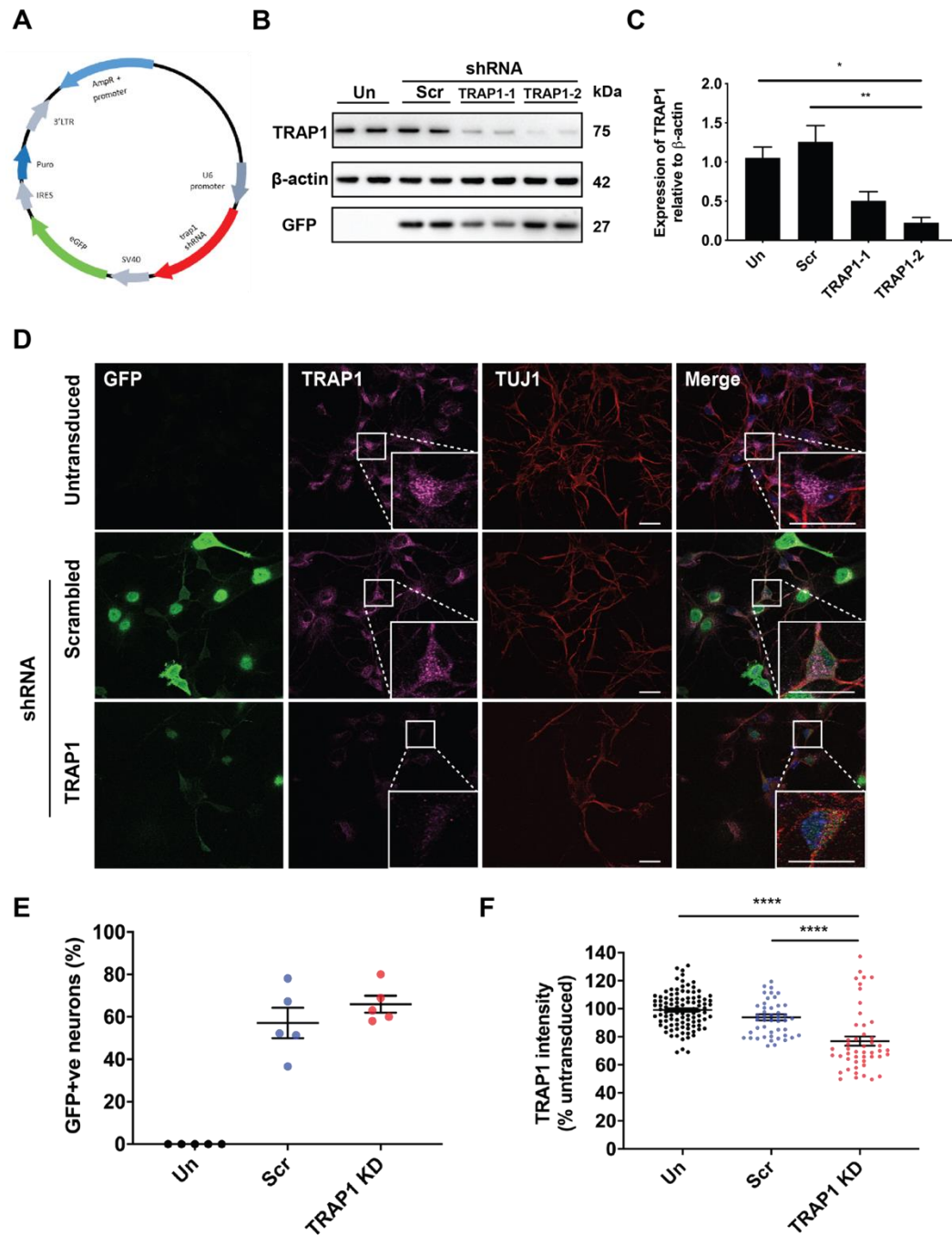


**Figure S1.** Characterisation of lentiviral overexpression of TRAP1. (A) Design of TRAP1 plasmid used for lentiviral overexpression of human TRAP1. Western blot for TRAP1 and GFP expression (B) and quantification (C) of lentiviral overexpression of TRAP1.  $n = 3$ . (D) Immunofluorescence images of primary motor neuron cultures for expression of GFP (green), HA (red) and TUJ1 (pink) treated with lentiviral expression constructs. (E) Quantification of GFP-positive neurons treated with lentiviral expression constructs.  $n = 6$ .

(F) Western blot for HA and GFP expression in primary motor neurons treated with lentiviral expression constructs. Scale bar: 20  $\mu$ m.



**Figure S2.** Lentivirally expressed TRAP1 localises to mitochondria. **(A)** Colocalisation of TRAP1 and the HA tag. **(B)** Colocalisation of the HA tag and TOM20. Scale bar: 20  $\mu$ m.



**Figure S3.** Characterisation of lentiviral shRNA knockdown of TRAP1. (A) Design of the shRNA plasmids used for lentiviral knockdown of TRAP1. Western blot for TRAP1 and GFP expression (B) and quantification (C) of lentiviral knockdown of TRAP1.  $n = 3$ . (D) Immunofluorescence images of primary motor neuron cultures for expression of GFP (green), HA (red) and TUJ1 (pink) treated with lentiviral shRNA constructs. (E) Quantification of GFP-positive neurons.  $n = 5$ . (F) Quantification of lentivirally transduced primary motor neuron cultures for TRAP1 fluorescence intensity.  $n = 3$ . 47–101 neurons. Scale bar: 20  $\mu\text{m}$ .